IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: J. Prudent *et al.*
Serial No.: Group No.:
Filed: Examiner:
Entitled: **INVASIVE CLEAVAGE OF NUCLEIC ACIDS**

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.10

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U S Postal Service as "Express Mail Post Office to Addressee" under Express Mail Label No EL 837 033 803 US in an envelope addressed to Assistant Commissioner for Patents, Washington, D C 20231

Dated: February 22, 2002 By: Mary Ellen Waite
Mary Ellen Waite

Sir or Madam:

Prior to the prosecution of the present case, please make the following deletions and additions. A clean version of the pending claims with instructions for entry pursuant to 37 C.F.R. §1.121 (c)(3) is included below. A version with markings to show changes made is provided at Appendix 1, in accordance with 37 C.F.R. §§ 1.121(b)(1)(iii). For the Examiner's convenience, the entire set of pending claims is provided at Appendix 2.

IN THE TITLE:

Please change the Title of the Invention from "Invasive Cleavage of Nucleic Acids" to --Nucleic Acid Detection Assays--.

IN THE SPECIFICATION

On page 1, please delete the paragraph between the Title of the Invention and the Field of the Invention and replace it with the following paragraph:

--The present application is a Continuation of co-pending U.S. Appln. Ser. No. 09/982,667, filed October 18, 2001, which is a continuation of U.S. Appln. Ser. No. 09/350,309, filed July 9, 1999, now U.S. Patent No. 6,348,314, which is a Divisional of U.S. Appln. Ser. No. 08/756,386, filed November 29, 1996, now U.S. Patent No. 5,985,557, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/682,853, filed July 12, 1996, now U.S. Patent No. 6,001,567, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/599,491, filed January 24, 1996, now U.S. Patent No. 5,846,717.

The invention was made with government support under Cooperative Agreement 70NANB5H1030 awarded by the Department of Commerce, National Institute of Standards and Technology, Advanced Technology Program and Grant No. DE-FG02-94ER81891 awarded by the Department of Energy. The Government has certain rights in the invention.--

IN THE CLAIMS:

Please cancel Claims 1-25.

Please add the following Claims:

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, comprising:

a) providing:

i) a cleavage agent;

ii) a source of target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein a first portion of said first oligonucleotide comprises at least one nucleotide analog and wherein said first portion is completely complementary to said first portion of said first target nucleic acid, and;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion,

wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

b) combining said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs and cleaves said first oligonucleotide to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

28. The method of Claim 26, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

29. The method of Claim 27, wherein said 3' terminal nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

30. The method of Claim 28, wherein said single nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

31. The method of Claim 26, wherein said target nucleic acid comprises a nucleotide analog.

32. The method of Claim 31, wherein said first region of said target nucleic acid comprises said nucleotide analog.

33. The method of Claim 26, wherein said cleavage of said cleavage structure cleaves said first oligonucleotide.

34. The method of Claim 27, wherein said cleavage of said first cleavage structure cleaves in said first portion of said first oligonucleotide.

35. The method of Claim 27, wherein said first oligonucleotide is cleaved 5' of a nucleotide analog.

36. The method of Claim 35, wherein said first oligonucleotide is cleaved adjacent to and 5' of a nucleotide analog.

37. The method of Claim 27, wherein said first oligonucleotide is cleaved 3' of a nucleotide analog.

38. The method of Claim 37, wherein said first oligonucleotide is cleaved adjacent to and 3' of a nucleotide analog.

39. The method of Claim 27, wherein said first oligonucleotide further comprises a second portion, wherein said second portion is 5' of said first portion, and wherein said second portion comprises at least one nucleotide analog.

40. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

41. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

42. The method of Claim 26, wherein said first oligonucleotide comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said cleavage structure comprises detection of an increase in fluorescence intensity.

43. The method of Claim 26, wherein said detecting the cleavage of said cleavage

structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

44. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

45. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

46. The method of Claim 26, wherein said target nucleic acid comprises synthetic nucleic acid.

47. The method of Claim 46, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

48. The method of Claim 47, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

49. The method of Claim 46, wherein said synthetic target nucleic acid comprises DNA.

50. The method of Claim 26, wherein said cleavage agent comprises an enzyme.

51. The method of Claim 50, wherein said enzyme comprises a DNA polymerase.

52. The method of Claim 51, wherein said DNA polymerase comprises a thermostable DNA polymerase.

53. The method of Claim 52, wherein said thermostable DNA polymerase is derived from an organism from genus Thermus.

54. The method of Claim 53, wherein said organism from genus Thermus is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

55. The method of Claim 50, wherein said enzyme comprises a 5' nuclease.

56. The method of Claim 50, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

57. The method of Claim 56, wherein said thermostable DNA polymerase modified to have reduced synthetic activity is derived from an organism from genus Thermus.

58. The method of Claim 57, wherein said organism from genus Thermus is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

59. A method of detecting a target nucleic acid, comprising:

a) providing:

i) a cleavage agent;

ii) a sample suspected of containing a target nucleic acid;

iii) a first oligonucleotide comprising a 5' portion complementary to a first region of said target nucleic acid; and

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said first or said second regions of said target nucleic acid comprises a nucleotide analog;

b) combining said cleavage agent, said sample suspected of containing a target nucleic acid, said first oligonucleotide and said second oligonucleotide

under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

60. A kit comprising:

i) a cleavage agent;

ii) a first oligonucleotide comprising a 5' portion complementary to a first region of a target nucleic acid; and

iii) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said target nucleic acid comprises a nucleotide analog.

61. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

62. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

63. The kit of Claim 54, wherein said kit further comprises a solid support.

64. The kit of Claim 63, wherein said first oligonucleotide is attached to said solid support.

65. The kit of Claim 63, wherein said second oligonucleotide is attached to said solid support.

66. The kit of Claim 60, wherein said cleavage agent comprises a structure-specific nuclease.

67. The kit of Claim 66, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

68. The kit of Claim 60, wherein said cleavage agent comprises a 5' nuclease.

69. The kit of Claim 68, wherein said 5' nuclease comprises a thermostable 5' nuclease.

70. The kit of Claim 66, wherein a portion of the amino acid sequence of said structure-specific nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

71. The kit of Claim 70, wherein said thermophilic organism is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

72. The kit of Claim 66, wherein said structure-specific nuclease comprises a FEN-1 endonuclease.

73. The kit of Claim 60, further comprising a buffer solution.

74. The kit of Claim 73, wherein said buffer solution comprises a source of divalent cations.

75. The kit of Claim 74, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

76. The kit of Claim 60, further comprising providing a third oligonucleotide

complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid.

77. The kit of Claim 60, further comprising a target nucleic acid.

78. The kit of Claim 77, further comprising a second target nucleic acid.

79. The kit of Claim 78, further comprising a third oligonucleotide comprising a 5' portion complementary to a first region of said second target nucleic acid.

80. The kit of Claim 79, wherein said 3' portion of said third oligonucleotide is covalently linked to said second target nucleic acid.

81. The kit of Claim 79, wherein said second target nucleic acid further comprises a 5' portion, wherein said 5' portion of said second target nucleic acid is said third oligonucleotide.

REMARKS

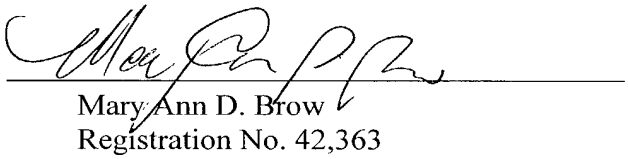
Claims 1-25 were in the Application as filed. Claims 1-25 have been cancelled without prejudice, and applicants reserve the right to prosecute the original claims (or similar claims) in the future. New claims 26-81 have been added. Support for the new claims is found throughout the Application. For example, examples of nucleotide analogs and modified nucleotides for use in the invention are described on page 30 at lines 27-30, and the use of such analogs in the oligonucleotides of the invention is described, *e.g.*, on page 11, lines 16-20. An example of target nucleic acids comprising nucleotide analogs is provided in Example 18 on page 152, lines 19-21. Additional support for the use of oligonucleotides and cleavage structures comprising nucleotide analogs is provided, for example, in Example 23, from page 173, line 23, to page 181, line 29. A schematic diagram of one example of such oligonucleotides and cleavage structures is provided in Figure 60A, with detailed diagrams of some embodiments of nucleotide analogs used in the invention provided in Figures 56-58. Additional support for cleavage 5' or 3' of a nucleotide analog is provided, for example, in the descriptions of oligonucleotides comprising uncleavable regions, *e.g.*, comprising thiolated nucleotides, on pages 34 and 122, with one

embodiment shown schematically in Figure 1A. Additional support for cleavage 3' of a base analog is provided, for example, in Figure 60A.

Support for the use of oligonucleotides attached to solid supports is provided, for example, on page 39, lines 25-26: "It is preferred that one of these oligonucleotides is provided on a solid support". A schematic representation of one embodiment of use of an oligonucleotide attached to a solid support is provided in Figure 1A.

Support for the claimed labels and detection methods is provided, *e.g.*, on page 28, lines 8-13 and on pages 57, line 26 to page 58, line 16. Additional support, *e.g.*, for oligonucleotides comprising fluorophors having quenched emissions, is provided, for example, on page 128, lines 19-21, reciting, "In this embodiment, the oligo contains two fluorescein labels whose proximity on the oligo causes their emission to be quenched." One embodiment of fluorophores having quenched emissions is shown schematically in Figure 27 of the specification.

Dated: February 22, 2002



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Appendix 1
Version With Markings To Show Changes Made
in Accordance with 37 C.F.R. § 1.121(b)(1)(iii)

IN THE TITLE:

Please change the Title of the Invention from "Invasive Cleavage of Nucleic Acids" to --Nucleic Acid Detection Assays--.

IN THE SPECIFICATION

On page 1, please delete the paragraph between the Title of the Invention and the Field of the Invention and replace it with the following paragraph:

--The present application is a Continuation of co-pending U.S. Appln. Ser. No. 09/350,309, filed July 9, 1999, which is a Divisional of U.S. Appln. Ser. No. 08/756,386, filed November 29, 1996, now U.S. Patent No. 5,985,557, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/682,853, filed July 12, 1996, now U.S. Patent No. 6,001,567, which is a Continuation-In-Part of U.S. Appln. Ser. No. 08/599,491, filed January 24, 1996, now U.S. Patent No. 5,846,717.

The invention was made with government support under Cooperative Agreement 70NANB5H1030 awarded by the Department of Commerce, National Institute of Standards and Technology, Advanced Technology Program and Grant No. DE-FG02-94ER81891 awarded by the Department of Energy. The Government has certain rights in the invention.--

In The Claims:

Please cancel Claims 1-25.

Please add the following claims:

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, comprising:

providing:

i) a cleavage agent;

ii) a source of target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein a first portion of said first oligonucleotide comprises at least one nucleotide analog and wherein said first portion is completely complementary to said first portion of said first target nucleic acid, and;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

b) combining said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs and cleaves said first oligonucleotide to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

28. The method of Claim 26, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

29. The method of Claim 27, wherein said 3' terminal nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

30. The method of Claim 28, wherein said single nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

31. The method of Claim 26, wherein said target nucleic acid comprises a nucleotide analog.

32. The method of Claim 31, wherein said first region of said target nucleic acid comprises said nucleotide analog.

33. The method of Claim 26, wherein said cleavage of said cleavage structure cleaves said first oligonucleotide.

34. The method of Claim 27, wherein said cleavage of said first cleavage structure cleaves in said first portion of said first oligonucleotide.

35. The method of Claim 27, wherein said first oligonucleotide is cleaved 5' of a nucleotide analog.

36. The method of Claim 35, wherein said first oligonucleotide is cleaved adjacent to and 5' of a nucleotide analog.

37. The method of Claim 27, wherein said first oligonucleotide is cleaved 3' of a nucleotide analog.

38. The method of Claim 37, wherein said first oligonucleotide is cleaved adjacent to and 3' of a nucleotide analog.

39. The method of Claim 27, wherein said first oligonucleotide further comprises a second portion, wherein said second portion is 5' of said first portion, and wherein said second portion comprises at least one nucleotide analog.

40. The method of Claim 26, wherein said detecting the cleavage of said cleavage

structure comprises detection of fluorescence.

41. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

42. The method of Claim 26, wherein said first oligonucleotide comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said cleavage structure comprises detection of an increase in fluorescence intensity.

43. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

44. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

45. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

46. The method of Claim 26, wherein said target nucleic acid comprises synthetic nucleic acid.

47. The method of Claim 46, wherein said synthetic target nucleic acid comprises an amplified nucleic acid.

48. The method of Claim 47, wherein said amplified nucleic acid is produced using a polymerase chain reaction.

49. The method of Claim 46, wherein said synthetic target nucleic acid comprises DNA.

50. The method of Claim 26, wherein said cleavage agent comprises an enzyme.

51. The method of Claim 50, wherein said enzyme comprises a DNA polymerase.

52. The method of Claim 51, wherein said DNA polymerase comprises a thermostable DNA polymerase.

53. The method of Claim 52, wherein said thermostable DNA polymerase is derived from an organism from genus Thermus.

54. The method of Claim 53, wherein said organism from genus Thermus is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

55. The method of Claim 50, wherein said enzyme comprises a 5' nuclease.

56. The method of Claim 50, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

57. The method of Claim 56, wherein said thermostable DNA polymerase modified to have reduced synthetic activity is derived from an organism from genus Thermus.

58. The method of Claim 57, wherein said organism from genus Thermus is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

59. A method of detecting a target nucleic acid, comprising:

a) providing:

i) a cleavage agent;

ii) a sample suspected of containing a target nucleic acid;

iii) a first oligonucleotide comprising a 5' portion complementary to a first region of said target nucleic acid; and

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said first or said second regions of said target nucleic acid comprises a nucleotide analog;

b) combining said cleavage agent, said sample suspected of containing a target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

60. A kit comprising:

i) a cleavage agent;

ii) a first oligonucleotide comprising a 5' portion complementary to a first region of a target nucleic acid; and

iii) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;
wherein at least one of said first oligonucleotide, said second oligonucleotide or said target nucleic acid comprises a nucleotide analog.

61. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

62. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

63. The kit of Claim 54, wherein said kit further comprises a solid support.

64. The kit of Claim 63, wherein said first oligonucleotide is attached to said solid support.

65. The kit of Claim 63, wherein said second oligonucleotide is attached to said solid support.

66. The kit of Claim 60, wherein said cleavage agent comprises a structure-specific nuclease.

67. The kit of Claim 66, wherein said structure-specific nuclease comprises a thermostable structure-specific nuclease.

68. The kit of Claim 60, wherein said cleavage agent comprises a 5' nuclease.

69. The kit of Claim 68, wherein said 5' nuclease comprises a thermostable 5' nuclease.

70. The kit of Claim 66, wherein a portion of the amino acid sequence of said structure-specific nuclease is homologous to a portion of the amino acid sequence of a thermostable DNA polymerase derived from a thermophilic organism.

71. The kit of Claim 70, wherein said thermophilic organism is selected from the

group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

72. The kit of Claim 66, wherein said structure-specific nuclease comprises a FEN-1 endonuclease.

73. The kit of Claim 60, further comprising a buffer solution.

74. The kit of Claim 73, wherein said buffer solution comprises a source of divalent cations.

75. The kit of Claim 74, wherein said divalent cation is selected from the group consisting of Mn^{2+} and Mg^{2+} ions.

76. The kit of Claim 60, further comprising providing a third oligonucleotide complementary to a third portion of said target nucleic acid upstream of said first portion of said first target nucleic acid.

77. The kit of Claim 60, further comprising a target nucleic acid.

78. The kit of Claim 77, further comprising a second target nucleic acid.

79. The kit of Claim 78, further comprising a third oligonucleotide comprising a 5' portion complementary to a first region of said second target nucleic acid.

80. The kit of Claim 79, wherein said 3' portion of said third oligonucleotide is covalently linked to said second target nucleic acid.

81. The kit of Claim 79, wherein said second target nucleic acid further comprises a 5' portion, wherein said 5' portion of said second target nucleic acid is said third oligonucleotide.

Appendix 2
Entire Set Of Pending Claims

26. A method for detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products, comprising:

a) providing:

i) a cleavage agent;

ii) a source of target nucleic acid, said target nucleic acid comprising a first region and a second region, said second region downstream of and contiguous to said first region;

iii) a first oligonucleotide, wherein a first portion of said first oligonucleotide comprises at least one nucleotide analog and wherein said first portion is completely complementary to said first portion of said first target nucleic acid, and;

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is completely complementary to said second region of said target nucleic acid;

b) combining said cleavage agent, said target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs and cleaves said first oligonucleotide to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

27. The method of Claim 26, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

28. The method of Claim 26, wherein said 3' portion of said second oligonucleotideconsists of a single nucleotide not complementary to said target nucleic acid.

29. The method of Claim 27, wherein said 3' terminal nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

30. The method of Claim 28, wherein said single nucleotide not complementary to said target nucleic acid comprises a nucleotide analog.

31. The method of Claim 26, wherein said target nucleic acid comprises a nucleotide analog.

32. The method of Claim 31, wherein said first region of said target nucleic acid comprises said nucleotide analog.

33. The method of Claim 26, wherein said cleavage of said cleavage structure cleaves said first oligonucleotide.

34. The method of Claim 27, wherein said cleavage of said first cleavage structure cleaves in said first portion of said first oligonucleotide.

35. The method of Claim 27, wherein said first oligonucleotide is cleaved 5' of a nucleotide analog.

36. The method of Claim 35, wherein said first oligonucleotide is cleaved adjacent to and 5' of a nucleotide analog.

37. The method of Claim 27, wherein said first oligonucleotide is cleaved 3' of a nucleotide analog.

38. The method of Claim 37, wherein said first oligonucleotide is cleaved adjacent to and 3' of a nucleotide analog.

39. The method of Claim 27, wherein said first oligonucleotide further comprises a second portion, wherein said second portion is 5' of said first portion, and wherein said second portion comprises at least one nucleotide analog.

40. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of fluorescence.

41. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection of mass.

42. The method of Claim 26, wherein said first oligonucleotide comprises a fluorophore having quenched emission, and wherein said detecting the cleavage of said cleavage structure comprises detection of an increase in fluorescence intensity.

43. The method of Claim 26, wherein said detecting the cleavage of said cleavage structure comprises detection selected from the group consisting of detection of radioactivity, luminescence, dye intercalation, fluorescence polarization, staining, or color.

44. The method of Claim 26, wherein said first oligonucleotide is attached to a solid support.

45. The method of Claim 26, wherein said second oligonucleotide is attached to a solid support.

46. The method of Claim 26, wherein said target nucleic acid comprises synthetic nucleic acid.

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50. The method of Claim 26, wherein said cleavage agent comprises an enzyme.

51. The method of Claim 50, wherein said enzyme comprises a DNA polymerase.

52. The method of Claim 51, wherein said DNA polymerase comprises a thermostable DNA polymerase.

53. The method of Claim 52, wherein said thermostable DNA polymerase is derived from an organism from genus Thermus.

54. The method of Claim 53, wherein said organism from genus Thermus is selected from the group consisting of Thermus aquaticus, Thermus flavus, and Thermus thermophilus.

55. The method of Claim 50, wherein said enzyme comprises a 5' nuclease.

56. The method of Claim 50, wherein said enzyme comprises a thermostable 5' nuclease derived from a thermostable DNA polymerase modified to have reduced synthetic activity.

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a) providing:

i) a cleavage agent;

ii) a sample suspected of containing a target nucleic acid;

iii) a first oligonucleotide comprising a 5' portion complementary to a first region of said target nucleic acid; and

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said first or said second regions of said target nucleic acid comprises a nucleotide analog;

b) combining said cleavage agent, said sample suspected of containing a target nucleic acid, said first oligonucleotide and said second oligonucleotide under reaction conditions such that at least said first portion of said first oligonucleotide is annealed to said first region of said target nucleic acid, and wherein at least said 5' portion of said second oligonucleotide is annealed to said second region of said target nucleic acid so as to create a cleavage structure, and wherein cleavage of said cleavage structure occurs to generate a non-target cleavage product; and

c) detecting the cleavage of said cleavage structure.

60. A kit comprising:

i) a cleavage agent;

ii) a first oligonucleotide comprising a 5' portion complementary to a first region of a target nucleic acid; and

iii) a second oligonucleotide comprising a 3' portion and a 5' portion, said 5' portion complementary to a second region of said target nucleic acid downstream of and contiguous to said first portion;

wherein at least one of said first oligonucleotide, said second oligonucleotide or said target nucleic acid comprises a nucleotide analog.

61. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

62. The kit of Claim 60, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

63. The kit of Claim 54, wherein said kit further comprises a solid support.

64. The kit of Claim 63, wherein said first oligonucleotide is attached to said solid support.

65. The kit of Claim 63, wherein said second oligonucleotide is attached to said solid support.

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portion complementary to a first region of said second target nucleic acid.

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